

Cell Specificity in DNA Binding and Repair of Chemical Carcinogens

by James A. Swenberg,* Douglas E. Rickert,*
Bonnie L. Baranyi† and Jay I. Goodman†

Many animal models for organ specific neoplasia have been developed and used to study the pathogenesis of cancer. Morphologic studies have usually concentrated on the response of target cells, whereas biochemical investigations have usually employed whole organ homogenates. Since hepatocytes comprise nearly 90% of the liver's mass and 70-80% of its DNA, alterations in DNA replication, covalent binding and DNA repair of nonparenchymal cells are usually obscured when whole organ homogenates are used. By utilizing cell separation methods, we have been able to demonstrate differences between hepatocyte and nonparenchymal cell replication, DNA damage and repair following exposure to a variety of hepatocarcinogen. Differences in removal of simple O⁶-alkylguanine and DNA replication correlate with cell specific carcinogenesis of simply alkylating agents. For several other procarcinogens, including 2-acetylaminofluorene and dinitrofluorene, cell specificity appears to reside primarily in the differential metabolic competence of hepatocytes and nonparenchymal cells. This results in greater covalent binding of the carcinogen to hepatocyte DNA, although the DNA adducts are removed at a similar rate in both cell types.

Introduction

It has long been recognized that many chemical carcinogens are tissue- or organ-specific (1, 2). Principal mechanisms thought to be responsible for this tropism include the absorption and distribution of the compound, site(s) of biotransformation, DNA binding and repair, and the route of excretion. In addition to chemicals exhibiting organotropism, however, most compounds are also cell-specific. That is, the chemical selectively induces tumors in specific cell populations within the target organ. Several examples include the induction of gliomas in brains of rats by nitrosamides (3); pancreatic islet cell tumors by streptozotocin (4); liver angiosarcomas by 1,2-dimethylhydrazine (SDMH) (5, 6), dimethylnitrosamine (DMN) (7) and vinyl chloride (8); and hepatocellular carcinomas by diethylnitrosamine (9, 10), 2-acetylaminofluorene (2-AAF) (10, 11) and dinitrofluorene (DNT) (12).

Several of the same parameters responsible for organ specificity are involved in cell-specific carcinogenesis. Included among these are differences in

metabolic competence for activating or inactivating carcinogens, selective effects on cell replication, differences in DNA repair, and cell specific enhancement or promotion. The ability to distinguish such differences between cell populations within the target organ requires that one be able to separate the different cell populations. Otherwise, differences present in smaller populations will be obscured by changes in the major cell type. For example, the liver is primarily composed of hepatocytes, bile duct cells, and sinusoidal lining cells composed of endothelial and Kupffer cells. As noted above, several chemical carcinogens induce neoplasms of only one of these cell types. If the chemical causes hepatocellular carcinomas, whole liver will probably provide a reasonable approximation, since 90% of the liver's mass is composed of hepatocytes (13). Conversely however, carcinogens that induce bile duct carcinomas or vascular tumors of the liver are probably not well approximated by whole liver, since bile duct and sinusoidal lining cells comprise only 10-20% of the liver's cells. Thus, selective increases in DNA replication and covalent binding or impaired DNA repair could go undetected.

Methods for Liver Cell Separation

Several methods are now available for separating hepatocytes and sinusoidal lining cells. High yields of sinusoidal lining cells can be obtained by pronase

*Chemical Industry Institute of Toxicology, P.O. Box 12137, Research Triangle Park, NC 27709.

†Department of Pharmacology and Toxicology, Center for Environmental Toxicology, Michigan State University, East Lansing, MI 48824.

digestion of hepatocytes in a mixed liver cell suspension (14) following collagenase perfusion of the liver using the method of Berry and Friend (15). If both hepatocytes and sinusoidal cells are being investigated, either elutriation centrifugation (16, 17) or differential low speed centrifugation (18-20) can be used. The sinusoidal cells can be further separated into Kupffer and endothelial cells by elutriation centrifugation (16). These procedures can be performed aseptically if tissue culture studies are required. The techniques provide relatively pure populations of viable hepatocytes (Fig. 1a), sinusoidal lining cells (Fig. 1b), endothelial cells (Fig. 1c) and Kupffer cells (Fig. 1d).

Factors Involved in Cell-Specific Carcinogenesis of Alkylating Agents

Exposure of rats to 1,2-dimethylhydrazine (SDMH) results in colon tumors (5, 21) or liver

tumors (5, 6), depending on the dosing regimen. Nearly 100% of male Fischer-344 rats exposed to 30 ppm SDMH via the drinking water (≈ 2 mg/kg/day) developed angiosarcomas of the liver (6). Half of these animals had metastatic angiosarcoma in their lungs. Hepatocellular carcinomas also occurred, but in only 40% of the rats. Using this regimen, we have examined cell-specific DNA alkylation, repair and replication during continuous exposure for up to 4 weeks (6). Similar amounts of 7-methylguanine (7MG) were formed in both hepatocytes and sinusoidal lining cells, suggesting that differential alkylation is not the mechanism for cell specificity. The concentrations of 7MG rose from initial one day values of 320 and 470 pmole/mg DNA in sinusoidal lining cells and hepatocytes respectively, to 970 and 1020 pmole/mg DNA on day 3 (Fig. 2A). A slight decline to 750 pmole 7MG/mg DNA was noted in both cell populations after 4 weeks of exposure. This decrease in steady-state levels of 7MG could be associated with either decreased metabolic activation or

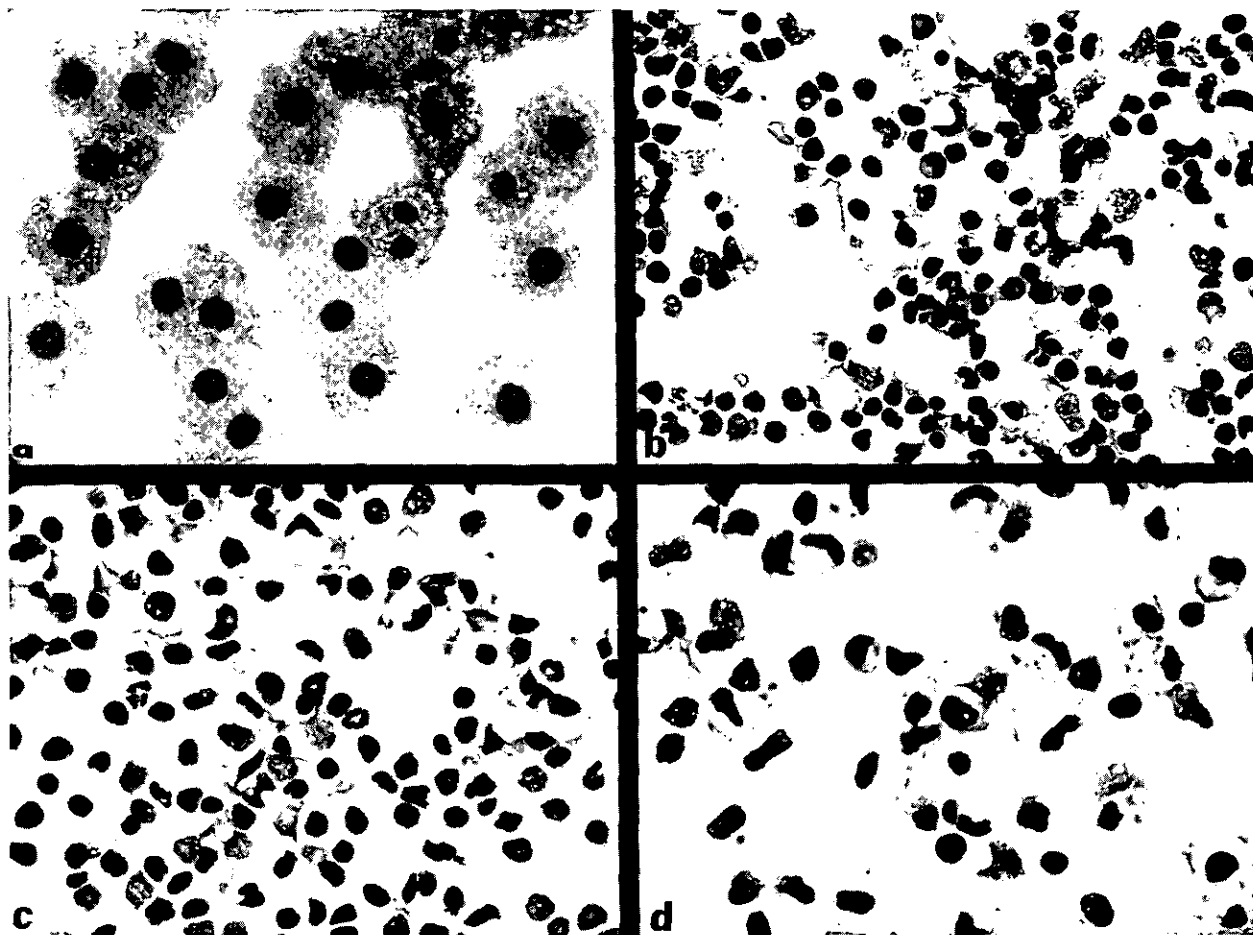


FIGURE 1. Photomicrographs of (a) hepatocytes, (b) sinusoidal lining cells, (c) endothelial cells and (d) Kupffer cells separated by centrifugal elutriation. H & E, $\times 285$.

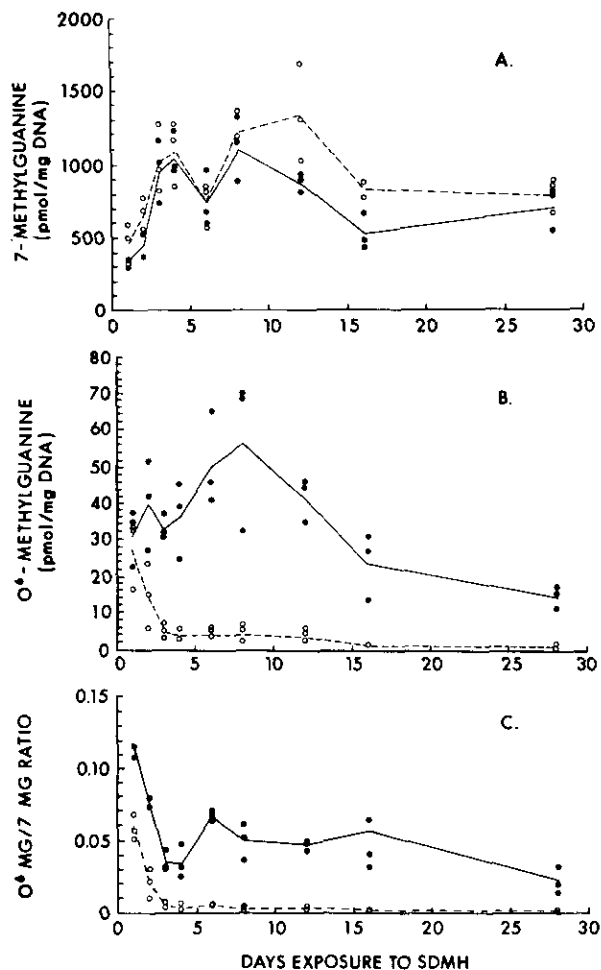


FIGURE 2. Normalized concentrations of (A) 7-methylguanine, (B) O⁶-methylguanine and (C) the O⁶/N-7 alkylation ratios in sinusoidal lining cells (●) and hepatocytes (○) exposed to 30 ppm dimethylhydrazine in the drinking water for intervals up to 28 days. Each data point represents one animal, while the curve connects the means of three animals at each time point (6). From Cancer Research with permission.

increased removal of 7MG. Support for the former is available from previous studies in mice (22) which demonstrated alterations in DMN metabolism, resulting in lower formation of 7MG. 7MG-DNA-glycosylase activity has not been measured in liver cells during continuous oral exposure to SDMH; however, it was not increased in whole liver homogenates following repeated administration of DMN to rats (23).

In marked contrast to 7MG, the concentration of the promutagenic DNA adduct, O⁶-methylguanine (O⁶MG), was similar in hepatocytes and sinusoidal lining cells only at day 1 of exposure (6). Thereafter, the sinusoidal cells accumulated O⁶MG, while the hepatocytes efficiently removed it (Fig. 2B). The

marked difference in the removal of O⁶MG was also evident when one compared the O⁶MG/7MG ratios. The sinusoidal lining cells showed an initial decrease, followed by a fairly constant ratio of 0.05. Hepatocytes exhibited an extremely rapid decrease in the O⁶MG/7MG ratios during the first 3 days and maintained a ratio of 0.005 for the last 25 days of a 4-week exposure (Fig. 2C).

We have recently measured the activity of the O⁶-alkylguanine alkyl acceptor protein in both cell populations following continuous exposure of rats to SDMH (24). A 2- to 3-fold enhancement of this alkyl acceptor protein occurred in hepatocytes after 3 days exposure to SDMH. This high activity was maintained in hepatocytes throughout the 4-week exposure. In contrast, the sinusoidal lining cells showed an initial decline in alkyl acceptor protein activity, followed by a return to control levels. Thus, the difference in alkylation at the O⁶ position of guanine can be explained by cell-specific differences in DNA repair.

Of equal importance, however, is the cell-specific mitogenic response to continuous SDMH exposure (25). The sinusoidal lining cells undergo a marked increase in *de novo* DNA synthesis, whereas a much smaller increase in *de novo* DNA synthesis occurs in hepatocytes (Fig. 3).

One can approximate the probability of mutation due to mispairing of O⁶MG in replicating DNA templates for each cell population by multiplying the concentration of O⁶MG, the amount of *de novo* DNA synthesis and the total amount of DNA at risk (Fig.

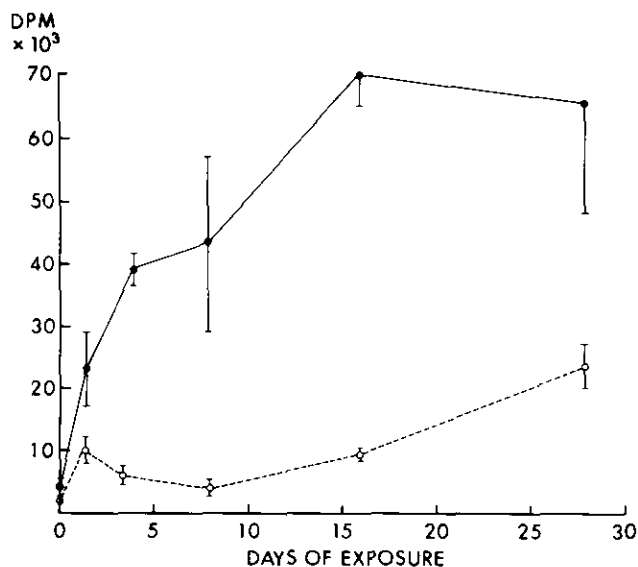


FIGURE 3. *De novo* DNA synthesis in (●) sinusoidal lining cells and (○) hepatocytes from rats exposed to dimethylhydrazine in the drinking water for various times (25). From Cancer Research with permission.

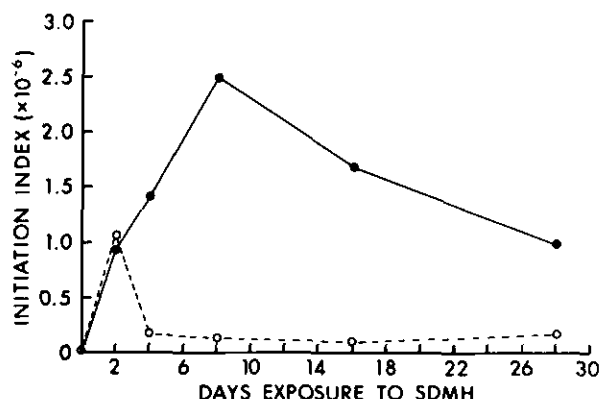


FIGURE 4. Initiation Index for SDMH exposure of rats represents the probability of mutation due to O⁶MG. Data points represent the product of O⁶MG concentration (6) and *de novo* DNA synthesis (25) per mg DNA times the amount of DNA at risk per cell population for (●) sinusoidal lining cells and (○) hepatocytes.

4). It is readily apparent that the sinusoidal lining cells have a greater probability for mutation than do hepatocytes. These data are consistent with carcinogenesis studies at the same dosing regimens (6).

Similar data have been obtained from mice exposed to 100 ppm DMN in their drinking water for up to 32 days (20). A progressive mitogenic response was evident in sinusoidal lining cells from 4 to 32 days of DMN exposure. Hepatocytes maintained a relatively constant elevated rate of *de novo* DNA synthesis from 4 to 32 days exposure. The sinusoidal lining cells had a progressive increase of O⁶MG from 2 to 32 days of DMN exposure, while hepatocytes maintained relatively constant low concentrations of O⁶MG. At 32 days, the sinusoidal lining cells had 12 times more O⁶MG per milligram DNA than hepatocytes. Thus, the probability of GC → AT transitions due to miscoding of O⁶MG is again much greater in sinusoidal lining cells, the target cells for DMN carcinogenesis in the mouse (26).

It must be emphasized that these data only reflect the contribution of O⁶MG to the initiation of carcinogenesis. The data must be fortified with the probability of mutation due to other forms of promutagenic DNA damage, since it is the sum of mutations due to all promutagenic lesions that is believed to comprise initiation. Cell specificity in carcinogenesis due to alkylating agents will depend on the ability of different cell populations to activate the carcinogen, their mitogenic response, their capacity to remove promutagenic DNA lesions and the nature of the alkylating agent. For instance, the major promutagenic lesion produced by SN, methylating agents is O⁶MG (27). It probably represents the principal lesion responsible for initiation in those cell types, such as liver sinusoidal cells, that

have low to moderate O⁶-alkylguanine alkyl acceptor protein activity (24). O-Alkylated pyrimidines, such as O² and O⁴-thymine, occur in greater proportion relative to O⁶-alkylguanine following exposure to ethylating agents (27). Since the O-alkylpyrimidines are removed from liver DNA more slowly (28, 29), they may represent the primary lesions responsible for initiation in those cell types, such as hepatocytes, that have high O⁶-alkylguanine alkyl acceptor protein activity.

Factors Involved in Cell-Specific Carcinogenesis of N-Substituted Aryl Compounds

The amount of information available on cell-specific factors involved in N-substituted aryl carcinogenesis is more limited than that available for alkylating agents. Hepatocytes represent the principal target cell of most hepatocarcinogenic N-substituted aryl compounds (30). Tumors of bile duct epithelium also occur, whereas tumors of sinusoidal lining cells are rare.

2-Acetylaminofluorene

2-Acetylaminofluorene (AAF) is a model hepatocarcinogen, inducing a high incidence of hepatocellular carcinomas. Multiple pathways exist for activation of AAF to a reactive electrophile. The importance of N-hydroxylation of AAF as the first step in the hepatocarcinogenic process was confirmed when it was found that N-hydroxyacetylaminofluorene (N-OH-AAF) was more potent than the parent compound in producing tumors of the liver, mammary gland, small intestine and ear duct of the rat when given in an equimolar dose (10). In addition, N-OH-AAF produced tumors at the site of administration, while AAF was locally inactive (10). Esterification of the N-hydroxy group of N-OH-AAF as a result of sulfotransferase or N,O-acetyltransferase activity, and subsequent formation of a reactive electrophile are steps in the metabolic pathway thought to be important in hepatic carcinogenesis following treatment of animals with AAF (10, 31-34).

We have utilized the cell separation methods referred to above to compare binding of carcinogen adducts to DNA of hepatocytes and sinusoidal lining cells following administration of equimolar doses of AAF or N-OH-AAF.

An assessment of the binding of [ring-³H]-AAF (1.8 μmole/100 g) to DNA of hepatocytes and sinusoidal lining cells following a single IP injection is presented in Figure 5. At the time of peak binding, 18 hours post-injection, the ratio of carcinogen adduct per milligram of hepatocyte DNA as compared

to sinusoidal lining cells was 2.7, indicating an initial quantitative selectivity of AAF for binding to DNA of hepatocytes ($p < 0.05$). After 3 days, 59% of the adducts remained in DNA of hepatocytes, and 50% of the adducts remained in DNA of the sinusoidal lining cells.

The results of analogous studies with [ring- ^3H]-N-OH-AAF are presented in Figure 6. Two hours after a single injection of [ring- ^3H]-N-OH-AAF (1.8 $\mu\text{mole}/100\text{ g}$), the time of peak DNA binding, there was no significant difference in the amount of carcinogen bound per milligram of hepatocyte and sinusoidal lining cell DNA. Three days later, adducts had been removed from DNA of both cell populations to a similar extent, i.e., 54% of the adducts remained in DNA of hepatocytes, and 57% of the adducts remained in DNA of the sinusoidal lining cells.

When these data are adjusted for the amount of

DNA at risk for the two cell populations, the results clearly show that the amount of carcinogen bound to the DNA of target cells, i.e., hepatocytes, is much greater (Table 1). This cell specificity is greatest for the procarcinogen, AAF, suggesting that hepatocytes have greater metabolic competence for N-hydroxylating AAF. Support for this hypothesis is provided by our results with N-OH-AAF, where similar amounts of covalent binding were present per milligram DNA. The total amount of N-OH-AAF bound to hepatocellular DNA versus sinusoidal lining cell DNA was considerably greater, however. Thus, cell specificity in N-OH-AAF binding to DNA would still favor the induction of hepatocellular tumors.

Previous studies of Tulp et al. reported greater binding of N-OH-AAF to hepatocyte nuclei than to stromal cell nuclei (35). Similar differential binding was present in DNA from these cells. The reason for the differing results is unknown; however, the studies may not be directly comparable since the studies of Tulp et al. utilized a different time point,

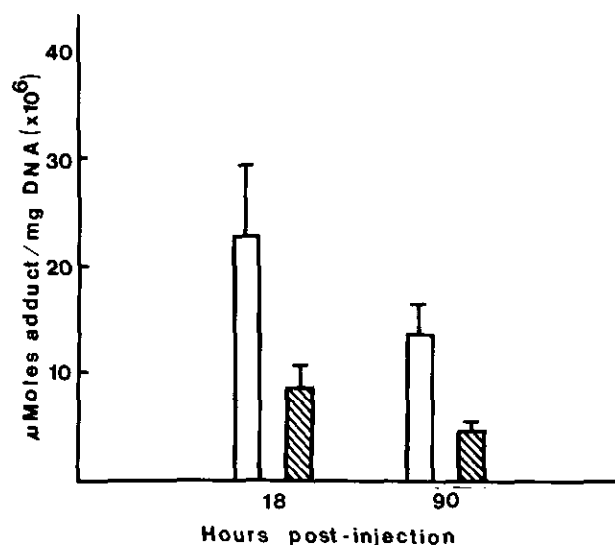


FIGURE 5. Binding of [ring- ^3H]AAF to DNA of hepatocytes (open bars) and sinusoidal lining cells (hatched bars). Following a single IP injection of [ring- ^3H]AAF (1.8 $\mu\text{mole}/100\text{ g}$), the amount of carcinogen bound/mg DNA was determined at the time of peak binding (18 hr after injection) and 3 days later. Results are expressed as the mean value from six rats. The standard error is represented by a bar. At 18 hr after injection the amount of carcinogen bound to the DNA of sinusoidal lining cells was significantly different ($p < 0.05$) from that bound to hepatocytes, as determined by Student's t -test at $p = 0.05$.

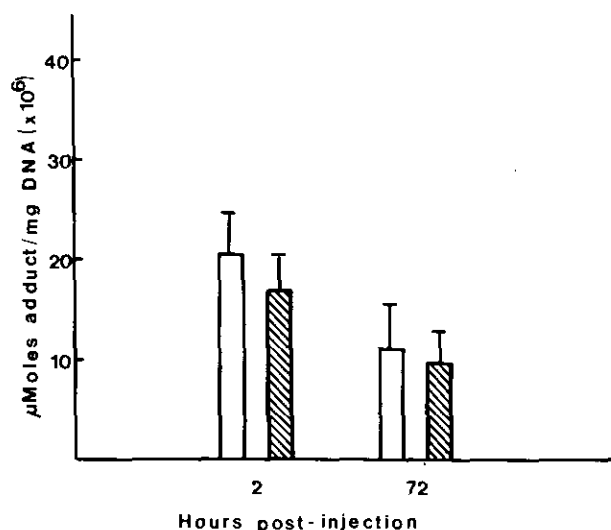


FIGURE 6. Binding of [ring- ^3H]N-OH-AAF to DNA of hepatocytes (open bars) and sinusoidal lining cells (hatched bars). Following a single IP injection of [ring- ^3H]N-OH-AAF (1.8 $\mu\text{mole}/100\text{ g}$), the amount of carcinogen bound per mg DNA was determined at the time of peak binding (2 hr after injection), and 3 days later. Results are expressed as the mean value from six rats. Standard error is represented by a bar.

Table 1. Total binding of AAF and N-OH-AAF to liver cell DNA.

Carcinogen	Time, hr	DNA binding, pmole/total DNA		Hepatocyte/sinusoidal Cell ratio
		Hepatocyte	Sinusoidal cell	
AAF	18	230 ± 43	12 ± 4	19.2
	90	95 ± 21	4 ± 1	23.8
N-OH-AAF	2	141 ± 32	17 ± 3	8.3
	72	76 ± 31	10 ± 3	7.6

a different cell separation procedure and a different DNA isolation method than we used. More recent studies from the same laboratories using AAF demonstrated similar amounts of the C-8 aminofluorene adduct in diploid stromal cells and tetraploid and octaploid hepatocytes, but much greater amounts of the C-8 acetylaminofluorene adduct in hepatocytes. Likewise, the total amount of covalent binding of AAF was greatest in hepatocytes, but removal of DNA adducts was similar in both cell populations (36).

Dinitrotoluene

Dinitrotoluene (DNT) is a high volume commodity chemical used in the production of explosives and polyurethane foams. Recent bioassays demonstrated that DNT was carcinogenic for laboratory animals (12); however, the target organ differed with different conditions. 2,6-DNT is a potent hepatocarcinogen in Fischer-344 rats (37), with initiating (38) and promoting (39) activity. It induces a 100% incidence of hepatocellular carcinomas in male rats within one year. Females also develop a high incidence of hepatocellular carcinomas; however, the latency period is somewhat longer. This sex difference is thought to be due to differences in the metabolism of DNT (40, 41). DNT is absorbed from the gastro-intestinal tract and transported to the liver, where it is metabolized to dinitrobenzyl alcohol and its glucuronide. In female rats, the glucuronide enters the general circulation and is primarily excreted in the urine, whereas in males it is excreted via the bile. The intestinal microflora hydrolyze the glucuronide and convert the aglycone to an as yet unidentified metabolite that is reabsorbed and transported to the liver. On this second pass through the liver, the unknown metabolite is covalently bound to hepatic macromolecules or is converted to a reactive species capable of covalent binding.

We have initiated studies to characterize the extent and persistence of 2,6-DNT covalent binding in hepatocytes and sinusoidal lining cells of male and female Fischer-344 rats in order to determine whether sex-dependent differences in DNA modification exist and whether covalent binding is greater in hepatocytes, the target cell for DNT carcinogenesis, than in the nontarget sinusoidal lining cell populations. Nine male and nine female Fischer-344 rats were dosed orally with 35 mg/kg ^3H -2,6-DNT (250 $\mu\text{Ci}/\text{rat}$). At 0.5, 4 or 7 days, the livers of three rats of each sex were perfused with collagenase and their hepatocytes and sinusoidal lining cells separated by differential centrifugation. DNA was isolated from hepatocytes of individual rats, whereas the sinusoidal lining cells from all three animals

were pooled prior to DNA isolation. The extent of covalent binding was determined by scintillation counting. DNA was measured using the diamino-benzoic acid method (42). Data expressed as DPM/mg DNA are shown in Figure 7, while total covalent binding per cell population is shown in Figure 8. DNA from male hepatocytes exhibited the greatest amount of covalent binding. DNA from female hepatocytes had slightly less initial binding. Covalently bound DNT was removed from hepatocyte DNA more rapidly during the first 4 days after exposure than during the subsequent 3 days. While the data on sinusoidal lining cells were limited due to pooling of samples, there was considerably less 2,6-DNT bound to their DNA and minimal evidence of DNA repair. For comparison, three germ-free male Fischer-344 rats were also examined for covalent binding to DNA 12 hours after exposure to ^3H -2,6-DNT. Hepatocellular DNA from the germ-free rats contained less than 10% of the amount of 2,6-DNT bound to DNA of conventional animals (Figs. 7 and 8).

When male rats were given weekly doses of ^3H -2,6-DNT (35 mg/kg, 250 $\mu\text{Ci}/\text{rat}$) and killed 7 days later, covalently bound radioactivity accumulated only in hepatocellular DNA (Fig. 9). Thus, the extent of covalent binding of ^3H -2,6-DNT exhibits a strong correlation in both cell and sex specificity. Additional investigations will be necessary to determine the nature of the covalently bound material. Preliminary data have demonstrated the presence of a single radioactive peak following HPLC separation of nucleosides.

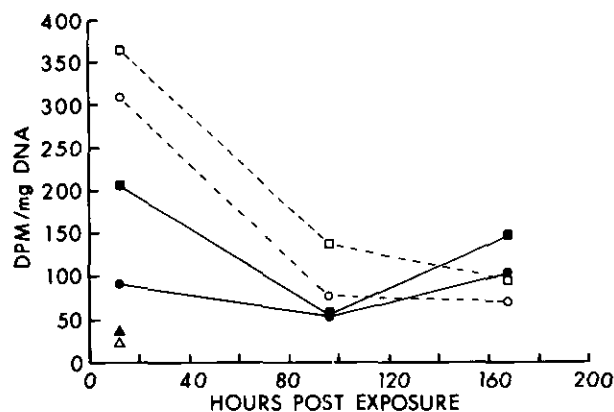


FIGURE 7. Covalent binding (DPM/mg DNA of ^3H -2,6-DNT (250 μCi , 35 mg/kg orally) in DNA from male (□, ■) and female (○, ●) rat hepatocytes (□, ○, △) and sinusoidal lining cells (■, ●, ▲). Male germ-free rats (△, ▲) have much lower amounts of covalent binding. Hepatocyte data points represent the mean of three rats, while sinusoidal lining cells from three rats were pooled for a single determination.

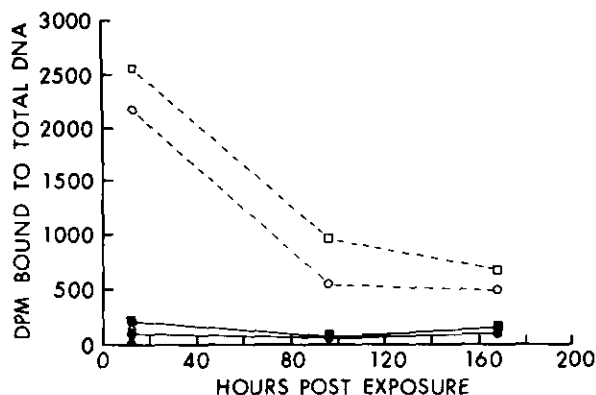


FIGURE 8. Data from Figure 7 expressed as total covalent binding to DNA for each cell population, using average yields of 1 mg DNA for sinusoidal lining cells and 7 mg DNA for hepatocytes.

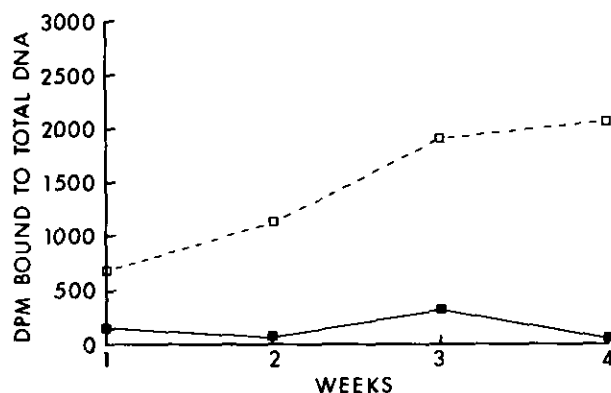


FIGURE 9. Cumulative covalent binding of ^3H -2,6-DNT to DNA. Male F-344 rats received 250 μCi (35 mg/kg) orally for 1, 2, 3 or 4 weeks. Hepatocytes (\square) or sinusoidal lining cells (\blacksquare) were isolated one week after the last dose. Data are expressed as DPM bound to the total DNA per cell population using pooled sinusoidal lining cells and the mean of three individual rats' hepatocytes.

Conclusions

Tissue and cell specificity in chemical carcinogenesis depends on a multiplicity of factors including the dose and route of exposure, absorption and distribution, the requirement for and site of biotransformation, chemical reactivity with DNA, the cellular capacity for repairing promutagenic DNA lesions and the amount of cell replication that takes place prior to such repair. Expression of these early events in chemical carcinogenesis is further complicated by exposure to promoting agents and toxins that selectively expand populations of initiated cells.

Cellular tropism for specific chemicals will be influenced to different degrees by these factors. For

example, high doses of alkylating agents will saturate the efficient hepatocyte O⁶-alkylguanine alkyl acceptor protein repair system (43), resulting in a greater likelihood for cell replication and resultant fixation of a GC \rightarrow AT transitions. Compensatory cell proliferation following selective toxicity is also dose-dependent. The magnitude of dose or concomitant exposure to other agents can also influence the site of biotransformation. Low oral doses of DMN are exclusively metabolized in the liver, whereas higher doses are activated in kidney (44). Selenium can selectively decrease SDMH metabolism in liver, resulting in increased systemic alkylation (45).

Metabolic differences between male and female rats result in greater covalent binding and subsequent tumor formation with AAF and DNT. Within the target organ, the metabolic competence of different cell populations affects the extent of DNA adduct formation as well as toxicity-induced compensatory cell proliferation. In most instances, this results in greater adduct formation in hepatocytes, both in terms of adducts per milligram DNA and adducts per total cell population. For chemicals such as AAF, removal of covalently bound carcinogen was similar in hepatocyte and sinusoidal lining cells, whereas major differences were apparent for removal of O⁶-alkylguanine. In the latter case, cell specificity becomes dependent on chemical reactivity with DNA. For example, the major promutagenic lesion induced by DNA by SN₁ methylating agents is O⁶MG (46). O⁶MG will therefore represent the major lesion responsible for initiation in those cell types, such as liver sinusoidal lining cells and neuroglial cells, that have low to moderate O⁶-alkylguanine alkyl acceptor protein activity (24). On the other hand, ethylating agents produce a greater proportion of O-alkylated promutagenic pyrimidines relative to O-alkylation of guanine. These O-alkylated pyrimidines are removed from hepatocyte DNA much more slowly (28, 29), providing prominent templates for mutations in cells that have high O⁶-alkylguanine alkyl acceptor protein activity and efficient removal of O⁶-alkylguanine.

Last, it is difficult to overestimate the importance of cell replication in chemical carcinogenesis. Mutations in a given cell population result from mis-coding of promutagenic damage in the parental strand during DNA replication. The mutations fixed in the daughter strand are amplified in succeeding generations through cell replication. This can occur at an enhanced rate due to selective cytotoxicity or specific promotion by the carcinogen or by exposure to other agents. Understanding the complex process of chemical carcinogenesis will require quantification of these individual components at the cellular level.

The authors are indebted to Ms. K. Billings and Ms. R. Long for their excellent technical assistance and to Ms. M. Bedell, Dr. J. Lewis and Dr. C. Lindamood for their many contributions to the work reviewed here. The investigations on AAF and N-OH-AAF were supported in part by USPHS Research Grant CA-30635 awarded by the National Cancer Institute.

REFERENCES

- Druckrey, H., Preussmann, R., Ivankovic, S., and Schmähl, D. Organotrope carcinogene Wirkungen bei 65 verschiedenen N-Nitroso-Verbindungen an BD-Ratten. *Z. Krebsforsch.* 69: 103-201 (1967).
- Magee, P. N., Organ specificity of chemical carcinogens. In: *Advances in Medical Oncology, Research and Education*, Vol. 1, Carcinogenesis (G. Margison, Ed.), Pergamon Press, New York, 1978, pp. 213-221.
- Swenberg, J. A. Chemical induction of brain tumors. In: *Advances in Neurology* (R. A. Thompson and J. R. Green, Eds.), Raven Press, New York, 1976, pp. 85-99.
- Swenberg, J. A. Unpublished data.
- Druckrey, H. Organospecific carcinogenesis in the digestive tract. In: *Topics in Chemical Carcinogenesis* (W. Nakahara, S. Takayama, T. Sugimura, and S. Odashima, Eds.), University Park Press, Tokyo, 1972, pp. 73-101.
- Bedell, M. A., Lewis, J. G., Billings, K. C., and Swenberg, J. A. Cell specificity in hepatocarcinogenesis: preferential accumulation of O⁶-methylguanine in target cell DNA during continuous exposure of rats to 1,2-dimethylhydrazine. *Cancer Res.* 42: 3079-3083 (1982).
- Lijinsky, W., and Reuber, M. D. Comparative carcinogenesis by some aliphatic nitrosamines in Fischer rats. *Cancer Letters*, 14: 297-302 (1981).
- Lee, C. C., Bhandari, J. C., Winston, J. M., House, W. B., Dixon, R. L., and Woods, J. S. Carcinogenicity of vinyl chloride and vinylidene chloride. *J. Toxicol. Environ. Health* 4: 15-30 (1978).
- Weisburger, J. H., Madison, R. M., Ward, J. M., Viguera, C., and Weisburger, E. K. Modification of diethylnitrosamine liver carcinogenesis with phenobarbital but not with immunosuppression. *J. Natl. Cancer Inst.*, 54: 1185-1188 (1975).
- Miller, E. C., Miller, J. A., and Hartmann, H. A. N-hydroxy-2-acetylaminofluorene: metabolite of 2-acetylaminofluorene with increased carcinogenic activity in the rat. *Cancer Res.* 21: 815-824 (1961).
- Albert, R. E., Burns, F. J., Bilger, L., Gardner, D., and Troll, W. Cell loss and proliferation induced by N-2-fluorenylacetylamine in the rat liver in relation to hepatoma induction. *Cancer Res.* 32: 2172-2177 (1972).
- Popp, J. A., and Leonard, T. B. The hepatocarcinogenicity of dinitrotoluenes. In: *Toxicity of Nitroaromatic Compounds*, Hemisphere Publ. Corp., New York, in press.
- Wisse, E., and Knook, D. L. The investigation of sinusoidal cells: a new approach to the study of liver function. In: *Progress in Liver Disease*, Vol. 6 (H. Popper and F. Schaffner, Eds.), Grune and Stratton, New York, 1979, pp. 153-171.
- Mills, D. M., and Zucker-Franklin, D. Electron microscopic study of isolated Kupffer cells. *Am. J. Pathol.* 54: 147. (1969).
- Berry, M. N., and Friend, D. S. High yield preparation of isolated rat liver parenchymal cells. *J. Cell Biol.* 43: 506-520 (1969).
- Knook, D. L., and Sleyster, E. Separation of Kupffer and endothelial cells by centrifugal elutriation. *Exp. Cell Res.* 99: 444-449. (1976).
- Lewis, J. G., and Swenberg, J. A. Differential repair of O⁶-methylguanine in DNA of rat hepatocytes and nonparenchymal cells. *Nature* 288: 185-187 (1980).
- Knook, D. L., and Sleyster, E. CH. Preparation and characterization of Kupffer cells from rat and mouse liver. In: *Kupffer Cells and Other Liver Sinusoidal Cells* (E. Wisse and D. L. Knook, Eds.), Elsevier/North-Holland Biomedical Press, Amsterdam, 1977, pp. 273-288.
- Doolittle, R. L., and Rickter, G. W. Isolation and culture of Kupffer cells and hepatocytes from single rat livers. *Lab. Invest.* 45: 558-566 (1981).
- Lindamood, C., Bedell, M. A., Billings, K. C., and Swenberg, J. A. Alkylation and *de novo* synthesis of liver cell DNA from C₃H mice during continuous DMN exposure. *Cancer Res.* 42: 4153-4157 (1982).
- Druckrey, H., Preussmann, R., Matzkies, F., and Ivankovic, S. Selective induction of intestinal cancer in rats with 1,2-dimethylhydrazine. *Naturwiss.* 54: 285-286 (1967).
- Den Engelse, L., and Emmelot, P., Effects of feeding the carcinogen dimethylnitrosamine on its metabolism and methylation of DNA in the mouse. *Chem.-Biol. Interactions* 4: 321-327 (1971).
- Margison, G. P., and Pegg, A. E. Enzymatic release of 7-methylguanine from methylated DNA by rodent liver extracts. *Proc. Natl. Acad. Sci. (U.S.)* 78: 861-865 (1981).
- Swenberg, J. A., Bedell, M. A., Billings, K. C., Umbenhauer, D. R., and Pegg, A. E. Cell specific differences in O⁶-alkylguanine-DNA repair activity during chronic carcinogen exposure. *Proc. Natl. Acad. Sci. (U.S.)* 79: 5499-5502 (1982).
- Lewis, J. G., and Swenberg, J. A., The effect of 1,2-dimethylhydrazine and diethylnitrosamine on cell replication and unscheduled DNA synthesis in target and non-target cell populations in rat liver following chronic administration. *Cancer Res.* 42: 89-92 (1982).
- Grasso, P., and Hardy, J. Strain difference in natural incidence and response to carcinogens. In: *Mouse Hepatic Neoplasia* (W. H. Butler, Ed.), Elsevier Publishing Corp., Amsterdam, 1975, pp. 111-132.
- Singer, B. Sites in nucleic acids reacting with alkylating agents of differing carcinogenicity or mutagenicity. *J. Toxicol. Environ. Health* 2: 1279-1295 (1977).
- Scherer, E., Timmer, A. P., and Emmelot, P. Formation by diethylnitrosamine and persistence of O⁶-ethylthymidine in rat liver DNA *in vivo*. *Cancer Letters* 10: 1-6 (1980).
- Singer, B., Spengler, S., and Bodell, W. J. Tissue-dependent enzyme mediated repair or removal of O-ethylpyrimidines and ethylpurines in carcinogen-treated rats. *Carcinogenesis* 2: 1069-1073 (1981).
- Weisburger, E. K., Russified, A. B., Homburger, F., Weisburger, J. H., Boger, E., VanDongen, C. G., and Chu, K. C. Testing of twenty-one environmental aromatic amines or derivatives for long-term toxicity or carcinogenicity. *J. Environ. Pathol. Toxicol.* 2: 325-356 (1978).
- Miller, E. C., and Miller, J. A. Searches for ultimate chemical carcinogens and their reactions with cellular macromolecules. *Cancer* 47: 2327-2345 (1981).
- Kriek, E., and Westra, J. G. Metabolic activation of aromatic amines and amides and interactions with nucleic acids. In: *Chemical Carcinogens and DNA*, Vol. 2, (P. L. Grover, Ed.), CRC Press, Boca Raton, FL, 1979, pp. 1-28.
- Miller, E. C., Miller, J. A., and Enomoto, M. The comparative carcinogenicities of 2-acetylaminofluorene and its N-hydroxy metabolite in mice, hamsters and guinea pigs. *Cancer Res.* 24: 2018-2031 (1964).
- Meerman, J. H. N., Beland, F. A., and Mulder, A. J. Role

- of sulfation in the formation of DNA adducts from N-hydroxy-2-acetylaminofluorene in rat liver *in vivo*. Inhibition of N-acetylated aminofluorene adduct formation by pentachlorophenol. *Carcinogenesis* 2: 413-416 (1981).
35. Tulp, A., Welagen, J. J. M. N., and Westra, J. G. Binding of the chemical carcinogen N-hydroxy-acetyl-aminofluorene to ploidy classes of rat liver nuclei as separated by velocity sedimentation at unit gravity. *Chem.-Biol. Interactions* 23: 293-303 (1978).
36. Westra, J. G., Visser, A., and Tulp, A. Binding and repair of 2-AAF adducts in distinct liver cell populations. *Environ. Health Perspect.* 49: 000-000 (1982).
37. Leonard, T. B. and Popp, J. A. Unpublished data.
38. Leonard, T. B. and Popp, J. A. Investigation of the carcinogenic initiation potential of dinitrotoluene (DNT): structure activity study. *Proc. Am. Assoc. Cancer Res.* 22: 82 (1981).
38. Leonard, T. B., and Popp, J. A. Dinitrotoluene promotion of diethylnitrosamine (DEN) initiated hepatocytes *in vivo*. *The Toxicologist* 2: 100 (1982).
39. Leonard, T. G., Lyght, O., and Popp, J. A. Structure-specific dinitrotoluene (DNT) promotion of initiated hepatocytes. *Proc. Am. Assoc. Cancer Res.* 23: 101 (1982).
40. Rickert, D. E., and Long, R. M. Metabolism and excretion of 2,4-dinitrotoluene in male and female Fischer-344 rats after different doses. *Drug Metab. Dispos.* 9: 226-232 (1981).
41. Bond, J. A., Medinsky, M. A., Dent, J. g., and Rickert, D. E. Sex-dependent metabolism and biliary excretion of ¹⁴C-2,4-dinitrotoluene in isolated perfused rat livers. *J. Pharmacol. Exptl. Therap.* 219: 598-603 (1981).
42. Hinegardner, R. J. An improved fluorometric assay for DNA. *Anal. Biochem.* 39: 197-201 (1971).
43. Pegg, A. E. Dimethylnitrosamine inhibits enzymatic removal of O⁶-methylguanine from DNA. *Nature* 274: 182-184 (1978).
44. Diaz-Gomez, M. I., Swann, P. F., and Magee, P. N. The absorption and metabolism in rats of small oral doses of dimethylnitrosamine. *Biochem. J.* 164: 164-500. (1977).
45. Harbach, P. R., and Swenberg, J. A. Effects on selenium on 1,2-dimethylhydrazine metabolism and alkylation. *Carcinogenesis* 2: 575-580 (1981).
46. Singer, B. The chemical effects of nucleic acid alkylation and their relation to mutagenesis and carcinogenesis. *Progr. Nucleic Acid Res. Med. Biol.* 15: 219-284 (1975).